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Synthesis and Cytotoxic Activity of New Pyrimido[1,2-c]quinazolines, [1,2,4]triazolo[4,3-c]quinazolines and (quinazolin-4-yl)-1*H*-pyrazoles Hybrids

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Abstract: New hybrid compounds with functionalized quinazoline derivatives and derived tricyclic compounds with varied condensed six and five-membered ring systems were synthesized from simple compounds. The 4-quinazolinimine, 2*H*-pyrimido[1,2-*c*] quinazolin-2-one, the pyrido[1,2-c]quinazoline and the [1,2,4]triazolo[4,3-c]quinazoline systems were synthesized starting form the key 4-azido compound and its isosteric analogue; 4-hydrazinyl derivative. Furthermore, a hybrid compound incorporating varied active motifs, the substituted tricyclic structural analog; 3-oxo-2-(quinazolin-4-yl)-2,3-dihydro-1*H*-pyrazole derivative, in which the pyrazole ring was attached to the quinazoline system via substitution at quinazoline-C^4, was also prepared. The synthesized compounds were studied for their cytotoxic activity against the human breast cancer (MCF-7) cell line. The tested compounds possess a high effect against the breast cancer cell line (MCF-7), and compounds 6b, 6e, 8, and 11 showed comparable results to those obtained for the reference drug doxorubicin. The results also showed the effect of substituents in the pyrimidoquinazoline and triazoloquinazoline ring systems on the cytotoxic activity. Additionally, the docking studies revealed that the compounds 6b, 6e, 8, and 11 bound well within the active sites of EGFRWT and EGFRT790M kinases and could be the lead molecules in the discovery of anticancer agents targeting inhibition of EGFRWT and EGFRT790M.

Keywords: quinazoline; pyrimido[1,2-*c*]quinazoline; [1,2,4]triazolo[4,3-*c*]quinazoline; pyrido[1,2-*c*]quinazoline; breast cancer; MCF-7.

1. Introduction

Cancer is believed as the main health threat for humans and a major life-threatening disease. It was considered the second leading cause of mortality, following cardiovascular diseases, and responsible for about 14.6% of worldwide deaths [1]. The discovery of novel potent and safe anticancer agents is the major serious aim in the medicinal chemistry research field due to the induced side effects of conventional non-selective cytotoxic chemotherapies, their systemic toxicity, and drug resistance. Molecular hybridization of small nitrogen heterocycles, leading to the formation of novel hybrid leads incorporating potent cores in one

molecule, has been revealed as an important strategy for designing new potent anticancer candidates [2,3]. Quinazolines constitute an important group of heterocycles that are well known for their multilateral and significant role in various biological activities, including anti-inflammatory, antimicrobial, antihyperlipidemic, antihypertensive, anticonvulsant, and anticancer activities [4-6]. Various quinazoline derivatives [7] and their analogs have been revealed for beneficial effects in various cancers. The pivotal role of quinazoline compounds has been referred to as selective and potent inhibitors for EGFR (epidermal growth factor receptor), a key target for cancer treatment [8,9], along with their remarkable antitumor activity [10-12]. Certain mono-substituted quinazoline derivatives (compounds I and II) were shown to exert a potential EGFR inhibition [13,14]. Also, the di-substituted quinazolines (III) based indolyl system showed good cytotoxicity activity against A-549, MCF-7, and HeLa cell lines, in addition to their nanomolar EGFR inhibitory activity [15].

The FDA (U.S. Food and Drug Administration) approved several EGFR, the most important of which are the quinazoline derivatives involving selective EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib [16-18] which were approved for NSCLC (non-small-cell lung cancer) treatment. Also, lapatinib is a reversible dual EGFR/HER2 TK inhibitor applied for breast cancer, mainly for the patient who revealed resistance to trastuzumab-based therapy [19,20]. Various new irreversible generations were discovered to overcome mutations of EGFR (L858R and T790M) associated with drug resistance [21,22]. Afatinib (Figure 1), the second-generation with dual EGFR/HER2 inhibition, had approval for the treatment of late-stage NSCLC patients with actively mutated EGFR by the FDA [23].

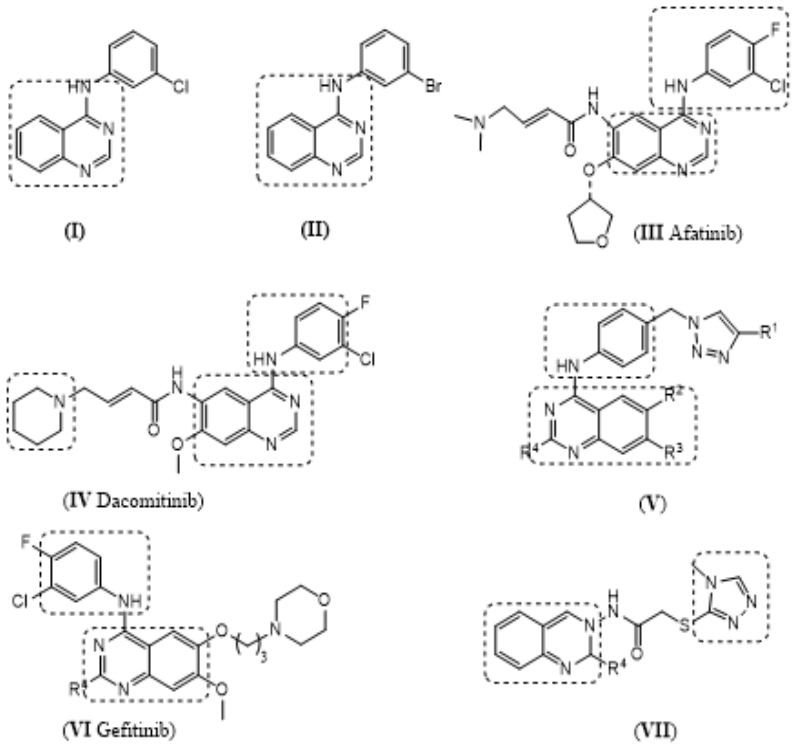


Figure 1. Substituted quinazolines of potent cytotoxicity against human cancer cells.

The substituted quinazoline compound, Dacomitinib (Figure 1), was detected for the NSCLC treatment [24,25]. Therefore, identifying potent irreversible EGFR/ HER2 dual inhibitors remains the core of research attention for future drugs [26]. The triazole-based quinazoline anticancer compound (V) [27] was designed and synthesized based on Afatinib (**3**) and its analog (IV). On the other hand, *in-vitro* studies showed that the triazole-quinazoline

hybrid derivative (VII) [28] had revealed anticancer activity against MCF-7 human cancer cells. The significances mentioned above for such heterocycles account for possible enhancements of their effect as anticancer candidates via incorporation with other potent motifs. The above facts and our interest in the synthesis and study of new anticancer candidates based on newly synthesized small heterocycles [29-33] promoted us, herein, to the synthesis and studying the anticancer activity of some new modified hybrid quinazoline derivatives with pyridine, pyrimidine and triazole cores. Moreover, a molecular docking study was achieved using MOE software version 2014.0901 to determine the possible binding interactions of the designed quinazolines with EGFRWT and EGFRT790M.

2. Materials and Methods

2.1. Chemistry.

A Kofler block melting point apparatus was applied to measure the newly synthesized quinazoline derivatives' melting points that were uncorrected. A Varian Gemini spectrometer (300 MHz, DMSO-d_6) was employed to record ^{1}H and ^{13}C NMR with an internal reference, tetramethylsilane (TMS). The coupling constants (J values) are given in hertz (Hz), and The chemical shifts are expressed in δ scale (ppm) relative to TMS as a reference. A Bruker-Vector22 spectrometer (Bruker, Bremen, Germany) was utilized to detect IR spectra (KBr). Mass spectra were achieved using a CC 2010 Shimadzu Gas chromatography instrument mass spectrometer (70 eV). Analytical thin-layer chromatography was done to examine the progress of the reactions using aluminum silica gel 60 F245 plates (Merck, Darmstadt, Germany). The microanalyses were performed at the microanalytical unit, Konstanz University, Germany. The cytotoxic activity of the newly synthesized derivatives was applied at the National Cancer Institute (NCI), Cairo, Egypt.

2.1.1. 4-Azido-7-bromo-6-chloroquinazoline (3).

A mixture of 7-bromo-6-chloroquinazolin-4-ol (2) (0.260 g, 1 mmol) and thionyl chloride (1.12 g, 10 mmol) was refluxed for 3 h, followed by removing the excess of thionyl chloride under decreased pressure. Then, the residue was treated with sodium azide (0.72 g, 1.1 mmol) in dry DMF (10 mL), stirring at 80 °C for 2 h. After that, the reaction mixture was triturated with water (5 mL) and left for 5 h at room temperature to afford a pale brown precipitate. Then, the precipitate was filtered off, washed with ether, and dried well to afford the azide compound (3) (0.28 g, 98%), m.p.: 213-214 ºC. IR (KBr, ν, cm-1): 2105 (N3), 1612 (C=N), 1570, 1425 (C=C Ar). 1H-NMR (CDCl3, 300 MHz): δ = 7.85 (s, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.88 (s, 1H, Ar-H). 13C-NMR (CDCl3, 75 MHz): δ = 125.5, 127.7, 129.3, 130.5, 145.8, 146.6, 155.3, 157.4 (Ar-Carbons) ppm. M.S-EI: m/z 282/284 (M)+. Anal. Calcd. for C8H3BrClN5 (284.50): C, 33.77; H, 1.06; N, 24.62; Found: C, 33.55; H, 1.00; N, 24.45%.

2.1.2. 7-Bromo-6-chloro-triphenylphosphine-4-quinazolinimine (4).

Triphenyl phosphine (0.28 g, 1.1 mmol) and the azide derivative (3) (0.57 g, 2 mmol) were dissolved in pyridine (4 mL) and left at room temperature for 1 h. Addition of water (15 mL) was followed by extraction with CH_2Cl_2 (3 x 15 mL), drying over Na_2SO_4, and filteration. The collected extracts were evaporated under reduced pressure to afford the phosphinimine product (4) (0.5 g, 97%), m.p.: 177-178 ºC. IR (KBr, ν, cm-1): 1609 (C=N), 1568, 1426 (C=C

Ar). 1H-NMR (DMSO-d6, 300 MHz) δ = 7.18-7.31 (m, 8H, Ar-H), 7.42-7.58 (m, 7H, Ar-H), 7.98 (s, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 8.97 (s, 1H, Ar-H). 13C-NMR (DMSO-d6, 75 MHz) δ = 122.11, 125.31, 126.07, 126.37, 126.52, 130.68, 131.41, 132.37, 154.42, 166.60 (Ar-Carbons). M.S-EI: *m/z* 517/519 = $(M)^+$. Anal. Calcd. for $C_{26}H_{18}BrClN_3P$ (518.77): C, 60.20; H, 3.50; N, 8.10; Found: C, 60.03; H, 3.33; N, 8.00%.

2.1.3. 7-Bromo-6-chloroquinazolin-4-amine (5).

The phosphinimine (4) (0.52 g, 1 mmol) in pyridine (5 mL) followed by addition of concentrated ammonium hydroxide (10 mL) and the solution was allowed to stand for 3 h. The solvents were removed in *vacuo* and the residue was purified by crystallization to give the corresponding amine (5) (0.22 g, 83%), m.p.: 290-292 ºC. IR (KBr, ν, cm^{-1}): 3275 cm^{-1} (NH_2), 1614 cm^{-1} (C=N), 1582, 1482 cm^{-1} (C=C Ar). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.61 (brs, 2H, NH_2, D_2O exchangeable), 7.87 (s, 1H, Ar-H), 8.07 (s, 1H, Ar-H), 8.46 (s, 1H, Ar-H), 7.78 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ = 114.22, 123.66, 125.61, 126.78, 128.73, 128.85, 133.04, 148.77, 155, 12, 161.87 (Ar-Carbons). M.S-EI: *m/z* 256/258 = $(M)^+$. Anal. Calcd. for $C_8H_5BrClN_3$ (258.50): C, 37.17; H, 1.95; N, 16.26; Found: C, 37.10; H, 1.88; N, 16.07%.

2.1.4. General procedures for preparation of compounds 6a-e.

A solution of quinazolinyl azide derivative (3) (0.285 g, 1 mmol), diethyl malonate, diethyl 2-methylmalonate, diethyl 2-ethylmalonate, diethyl 2-butylmalonate or diethyl 2-phenylmalonate (1 mmol), and diphenyl ether (5 mL) was refluxed for 1 h. Diethyl ether (10 mL) was added with strong stirring, and the products were collected by filtration to afford the pyrimidoquinazoline derivatives (6a-e), respectively, in 63-82% yields.

2.1.4.1. 7-Bromo-6-chloro-4-hydroxy-2H-pyrimido[1,2-c]quinazolin-2-one (6a).

Yield 0.21 g (63%), m.p.: 279-280 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 6.99 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.32 (s, 1H, Ar-H), 9.82 (brs, 1H, OH, D_2O exchangeable). M.S-EI: *m/z* 324/326 = $(M)^+$. Anal. Calcd. for $C_{11}H_5BrClN_3O_2$ (326.53): C, 40.46; H, 1.54; N, 12.87; Found: C, 40.32; H, 1.42; N, 12.66%.

2.1.4.2. 7-Bromo-6-chloro-4-hydroxy-3-methyl-2H-pyrimido[1,2-c]quinazolin-2-one (6b).

Yield 0.24 g (70%), m.p.: 295-297 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 2.34 (s, 3H, CH_3), 7.73 (s, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 8.31 (s, 1H, Ar-H), 9.93 (brs, 1H, OH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ = 8.57 (CH_3), 94.51, 112.28, 119.52, 120.09, 125.03, 127.76, 128.11, 136.22, 144.00, 150.98, 165.29 (Ar-Carbons). M.S-EI: *m/z* 338/340 = $(M)^+$. Anal. Calcd. for $C_{12}H_7BrClN_3O_2$ (340.56): C, 42.32; H, 2.07; N, 12.34; Found: C, 42.20; H, 2.00; N, 12.25%.

2.1.4.3. 7-Bromo-6-chloro-3-ethyl-4-hydroxy-2H-pyrimido[1,2-c]quinazolin-2-one (6c).

Yield 0.29 g (82%), m.p.: 240-242 ºC. IR (KBr, ν, cm-1): 3360 cm-1 (OH), 1674 cm-1 (C=O). 1H-NMR (DMSO-d6, 300 MHz) δ = 1.04 (t, 3H, J = 4.5 Hz, 3H, CH3), 3.39-3.44 (q, 2H, J = 4.4 Hz, CH2), 7.91 (s, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 8.48 (s, 1H, Ar-H), 9.32 (s, 1H, OH, D2O exchangeable). 13C-NMR (DMSO-d6, 75 MHz) δ = 12.53 (CH3), 16.41 (CH2),

100.64, 112.38, 120.09, 127.79, 128.06, 134.21, 136.15, 138.00, 144.07, 146.95, 163.40, 165.08 (Ar-Carbons). M.S-EI: *m/z* 352/354 = [M^+]. Anal. Calcd. for $C_{13}H_9BrClN_3O_2$ (354.59): C, 44.03; H, 2.56; N, 11.85; Found: C, 43.88; H, 2.33; N, 11.67%.

2.1.4.4. 7-Bromo-3-butyl-6-chloro-4-hydroxy-2H-pyrimido[1,2-c]quinazolin-2-one (6d).

Yield 0.31 g (80%), m.p.: 285-287 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 1.00 (t, 3H, *J* = 4.5 Hz, 3H, CH_3), 1.27 (m, 2H, CH_2), 1.34 (m, 1H, CH_2), 4.38 (m, 2H, CH_2), 7.78 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.39 (s, 1H, Ar-H), 9.32 (brs, 1H, OH, D_2O exchangeable). M.S-EI: *m/z* 380/382 = $(M)^+$. Anal. Calcd. for $C_{15}H_{13}BrClN_3O_2$ (382.64): C, 47.08; H, 3.42; N, 10.98; Found: C, 46.96; H, 3.32; N, 10.82%.

2.1.4.5. 7-Bromo-6-chloro-4-hydroxy-3-phenyl-2H-pyrimido[1,2-c]quinazolin-2-one (6e).

Yield 0.33 g (81%), m.p.: 275-277 ºC. IR (KBr, ν, cm^{-1}): 3450 cm^{-1} (OH), 1676 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.40-7.54 (m, 2H, Ar-H), 7.54-7.56 (m, 3H, Ar-H), 7.81 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 8.44 (s, 1H, Ar-H), 9.38 (brs, 1H, OH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ = 99.44, 119.85, 127.53, 127.88, 129.04, 130.51, 130.59, 132.45, 134.64, 138.18, 144.43, 147.90, 158.48, 164.85 (Ar-Carbons). M.S-EI: *m/z* 400/402 = $(M)^+$. Anal. Calcd. for $C_{17}H_9BrClN_3O_2$ (402.63): C, 50.71; H, 2.25; N, 10.44; Found: C, 50.62; H, 2.17; N, 10.31%.

2.1.5. 7-Bromo-6-chloro-4-hydrazinylquinazoline (7).

A mixture of (**2**) (0.26 g, 1 mmol) and thionyl chloride (1.12 g, 10 mmol) was refluxed for 3 h followed by removing the excess of thionyl chloride under decreased pressure. Then, the residue was treated with hydrazine hydrate (0.08 g, 1.5 mmol) and refluxed for 5 h. finally, the solvent was removed under reduced pressure to afford yellow powder from the corresponding 4-hydrazinylquinazoline (**7**) (0.23 g, 85%), m.p.: 180-182 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 4.90 (brs, 2H, NH_2, D_2O exchangeable), 7.83 (s, 1H, Ar-H), 8.16-8.25 (s, 2H, Ar-H), 9.61 (brs, 1H, NH, D_2O exchangeable). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ = 114.10, 122.19, 125.40, 127.28, 131.97, 148.41, 154.43, 155.39 (Ar-Carbons). M.S-EI: *m/z* 271/273 = $(M)^+$. Anal. Calcd. for $C_8H_6BrClN_4$ (273.52): C, 35.13; H, 2.21; N, 20.48; Found: C, 35.02; H, 2.11; N, 20.32%.

2.1.6. 7-Bromo-6-chloro [1,2,4]triazolo[4,3-*c*]quinazoline (8).

A mixture of (**7**) (0.274 g, 1 mmol), formic acid (0.046 g,1 mmol), and triethyl orthoformate (0.148 g, 1 mmol) was refluxed for 8 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (**8**) as yellow powder (0.21 g, 75%), m.p.: 110-112 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.79 (s, 1H, Ar-H), 8.06 (s, 1H, Ar-H), 8.86 (s, 1H, Ar-H), 9.16 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ = 116.40, 122.76, 123.17, 128.30, 128.45, 129.11, 129.33, 131.75, 132.22, 136.96, 137.35, 139.08, 146.06, 154.36 (Ar-Carbons). M.S-EI: *m/z* 281/283 = $(M)^+$. Anal. Calcd. for $C_9H_4BrClN_4$ (283.51): C, 38.13; H, 1.42; N, 19.76; Found: C, 38.00; H, 1.32; N, 19.63%.

2.1.7. 7-Bromo-6-chloro-3-methyl-[1,2,4]triazolo[4,3-c]quinazoline (9).

A mixture of (7) (0.274 g, 1 mmol), acetic acid (0.06 g, 1 mmol), and triethyl orthoformate (0.148 g, 1 mmol) was refluxed for 4 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (9) as pale yellow powder (0.21 g, 71%), m.p.: 210-212 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 2.83 (s, 3H, CH_3), 7.83-8.00 (m, 2H, Ar-H), 9.03 (s, 1H, Ar-H). M.S-EI: *m/z* 295/297 = $(M)^+$. Anal. Calcd. for $C_{10}H_6BrClN_4$ (297.54): C, 40.37; H, 2.03; N, 18.83; Found: C, 40.22; H, 1.93; N, 18.76%.

2.1.8. 7-Bromo-6-chloro-3-phenyl-[1,2,4]triazolo[4,3-*c*]quinazoline (10).

A mixture of (**7)** (0.274 g, 1 mmol), benzoyl chloride (0.14 g, 1 mmol) in pyridine (10 mL), and triethyl orthoformate (0.148 g, 1 mmol) was refluxed for 8 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give give (10) as brown crystals (0.25 g, 70%), m.p.: 178-180 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.50-7.66 (m, 3H, Ar-H), 7.84-7.95 (m, 3H, Ar-H), 8.17 (s, 1H, Ar-H), 8.98 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6, 75 MHz) δ = 117.51, 123.86, 127.00, 127.40, 128.29, 128.50, 129.05, 129.73, 130.23, 130.68, 131.51, 132.27, 138.96, 142.36, 150.73, 163.53 (Ar-Carbons). M.S-EI: *m/z* 357/359 = $(M)^+$. Anal. Calcd. for $C_{15}H_8BrClN_4$ (359.61): C, 50.10; H, 2.24; N, 15.58; Found: C, 50.00; H, 2.19; N, 15.44%.

2.1.9. 8-Amino-3-bromo-2-chloro-8*H*-pyrido[1,2-c]quinazoline-9-carbonitrile (11).

A mixture of (**7)** (0.274 g, 1 mmol), 2-(ethoxymethylene)malononitrile (0.122 g, 1 mmol), glacial acetic acid (10 mL), in ethanol (10 mL) was refluxed for 5 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (11) as pale yellow crystals (0.24 g, 70%), m.p.: 281-283 ºC. IR (KBr, ν, cm^{-1}): 3427 cm^{-1} (NH_2), 2216 cm^{-1} (C≡N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 4.60 (s, 1H, C-H), 7.15-7.20 (m, 2H, Ar-H), 7.45-7.53 (m, 2H, Ar-H), 7.70 (brs, 2H, NH_2, D_2O exchangeable), 8.57 (s, 1H, Ar-H). M.S-EI: *m/z* 334/336 = $(M)^+$. Anal. Calcd. for $C_{12}H_7BrClN_5$ (336.57): C, 42.82; H, 2.10; N, 20.81; Found: C, 42.66; H, 2.00; N, 20.67%.

2.1.10. 3-Bromo-2-chloro-8-oxo-8*H*-pyrido[1,2-c]quinazoline-9-carbonitrile (12).

A mixture of (**7)** (0.274 g, 1 mmol), ethyl 2-cyano-3-ethoxyacrylate (0.17 g, 1 mmol), glacial acetic acid (10 mL), in ethanol (10 mL) was refluxed for 4 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (12) as pale yellow crystals (0.25 g, 75%), m.p.: 267-269 ºC. IR (KBr, ν, cm^{-1}): 2211 cm^{-1} (C≡N), 1651 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.56 (s, 1H, Ar-H), 7.66 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 9.17 (s, 1H, Ar-H). M.S-EI: *m/z* 333/335 = $(M)^+$. Anal. Calcd. for $C_{12}H_4BrClN_4O$ (335.54): C, 42.95; H, 1.20; N, 16.70; Found: C, 42.82; H, 1.10; N, 16.50%.

2.1.11. Ethyl 3-bromo-2-chloro-8-oxo-8H-pyrido[1,2-c]quinazoline-9-carboxylate (13).

A mixture of (**7)** (0.274 g, 1 mmol), diethyl 2-(ethoxymethylene)malonate (0.22 g, 1 mmol), glacial acetic acid (10 mL), in ethanol (10 mL) was refluxed for 4 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and

recystallized from ethanol to give (13) as pale yellow crystals (0.33 g, 85%), m.p.: 230-232 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 1.30 (t, 3H, J = 6.5 Hz, CH_3), 4.30 (q, 2H, J = 6.5 Hz, CH_2), 5.99 (d, 1H, J = 5.5 Hz, Ar-H), 7.20 (s, 1H, Ar-H), 7.50-7.55 (m, 2H, Ar-H), 8.80 (d, 1H, J = 5.5 Hz, Ar-H). M.S-EI: m/z = 380/382 $(M)^+$. Anal. Calcd. for $C_{14}H_9BrClN_3O_3$ (382.60): C, 43.95; H, 2.37; N, 10.98; Found: C, 43.79; H, 2.17; N, 10.80%.

2.1.12. 5-Amino-7-bromo-6-chloro-1-(quinazolin-4-yl)-1H-pyrazole-4-carbonitrile (14).

A mixture of (**7**) (0.274 g, 1 mmol), 2-(ethoxymethylene)malononitrile (0.122 g, 1 mmol) in ethanol (10 mL) was refluxed for 5 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (14) as pale yellow powder (0.29 g, 83%), m.p.: 247-249 ºC. IR (KBr, ν, cm^{-1}): 3345 cm^{-1} (NH_2), 2215 cm^{-1} (C≡N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.47 (s, 1H, Ar-H), 8.04 (s, 1H, Ar-H), 8.24 (s, 1H, Ar-H), 9.15-9.25 (brs, 3H, Ar-H, NH_2). M.S-EI: m/z 347/349 = $(M)^+$. Anal. Calcd. for $C_{12}H_6BrClN_6$ (349.57): C, 41.23; H, 1.73; N, 24.04; Found: C, 41.09; H, 1.67; N, 23.96%.

2.1.13. 7-Bromo-6-chloro-3-oxo-2-(quinazolin-4-yl)-2,3-dihydro-1H-pyrazole-4-carbonitrile (15).

A mixture of (**7**) (0.274 g, 1 mmol), ethyl 2-cyano-3-ethoxyacrylate (0.169 g, 1 mmol) in ethanol (10 mL) was refluxed for 7 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (15) as pale yellow crystals (0.28 g, 81%), m.p.: 218-220 ºC. IR (KBr, ν, cm^{-1}): 3345 cm^{-1} (NH), 2215 cm^{-1} (C≡N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 7.17 (s, 1H, Ar-H), 8.05 (s, 2H, Ar-H), 8.44 (s, 1H, Ar-H), 9.15 (s, 1H, NH). M.S-EI: m/z 348/350 = $(M)^+$. Anal. Calcd. for $C_{12}H_5BrClN_5O$ (350.56): C, 41.11; H, 1.44; N, 19.98; Found: C, 41.03; H, 1.34; N, 19.86%.

2.1.14. Ethyl 7-bromo-6-chloro-3-oxo-2-(quinazolin-4-yl)-2,3-dihydro-1H-pyrazole-4-carboxylate (16).

A mixture of (**7**) (0.274 g, 1 mmol), diethyl 2-(ethoxymethylene)malonate (0.216 g, 1 mmol) in ethanol (10 mL) was refluxed for 5 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (16) as pale yellow crystals (0.31 g, 78%), m.p.: 232-234 ºC. IR (KBr, ν, cm^{-1}): 3182 cm^{-1} (NH), 1714 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 1.23 (t, 3H, J = 5.5 Hz, CH_3), 4.17 (q, 2H, J = 5.5 Hz, CH_2), 7.43 (s, 1H, Ar-H), 7.84 (s, 1H, Ar-H), 8.09 (s, 1H, Ar-H), 8.42 (s, 1H, Ar-H), 11.90 (brs, 1H, NH, D_2O exchangeable). M.S-EI: m/z = 395/397 $(M)^+$. Anal. Calcd. for $C_{14}H_{10}BrClN_4O_3$ (397.61): C, 42.29; H, 2.53; N, 14.09; Found: C, 42.18; H, 2.44; N, 14.00%.

2.1.15. 7-Bromo-6-chloro-3-methyl-1-(quinazolin-4-yl)-1H-pyrazol-5(4H)-one (18).

A mixture of (**7**) (0.274 g, 1 mmol), ethyl acetoacetate (0.13 g, 1 mmol), glacial acetic acid (10 ml), in ethanol (10 ml) was refluxed for 4 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (18) as pale yellow crystals (0.22 g, 66%), m.p.: 170-172 ºC. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 1.95 (s, 3H, CH_3), 3.00 (s, 2H, CH_2), 7.93 (s, 1H, Ar-H), 8.15 (s, 1H, Ar-H), 8.99 (s,

1H, Ar-H). M.S-EI: *m/z* = 337/339 $(M)^+$. Anal. Calcd. for $C_{12}H_8BrClN_4O$ (339.58): C, 42.44; H, 2.37; N, 16.50; Found: C, 42.30; H, 2.28; N, 16.41%.

2.1.16. 7-Bromo-6-chloro-4-(3,5-dimethyl-1H-pyrazol-1-yl)quinazoline (19).

A mixture of **7** (0.274 g, 1 mmol), acetyl acetone (0.10 g, 1 mmol), glacial acetic acid (10 mL), in ethanol (10 mL) was refluxed for 5 h. Then, the obtained mixture was allowed to cool to room temperature. The formed precipitate was filtered and recystallized from ethanol to give (19) as pale yellow crystals (0.26 g, 77%), m.p.: 251-253 ºC. IR (KBr, ν, cm^{-1}): 1612 cm^{-1} (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ = 1.91 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 4.98 (s, 1H, H-4 pyrazole), 7.90 (s, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 9.24 (s, 1H, Ar-H). M.S-EI: *m/z* = 335/337 $(M)^+$. Anal. Calcd. for $C_{13}H_{10}BrClN_4$ (337.60): C, 46.25; H, 2.99; N, 16.60; Found: C, 46.13; H, 2.81; N, 16.50%.

2.2. Cytotoxicity assay.

2.2.1. Measurement of potential cytotoxicity by SRB assay.

Some of the newly synthesized compounds have been evaluated for their Potential Cytotoxicity testing against breast cancer (MCF-7) using the method of Skehan and Storeng [34]. At first, cells were plated in a 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the screened compounds to allow cell attachment to the plate's wall. The screened compounds with different concentrations (0, 1, 2.5, 5, and 10 µg/ml) were added to the cell monolayer triplicate wells and were prepared for each individual dose. Then, the monolayer cells with the screened compounds were incubated for 48 h at 37°C and 5 % CO_2., The cells were fixed after 48 h, washed, and stained with Sulfo-Rhodamine-B stain. After that, the excess stain was removed through washing with acetic acid and recovered with Tris EDTA buffer. Finally, color Intensity was measured in an ELISA reader. The relation between drug concentration and the surviving fraction is plotted to have the survival curve of each tumor cell line after the specific compound. The IC_{50} percent control of infected and uninfected response values was calculated for the various active compounds reported in Table 1. Doxorubicin (DOX) was used as a positive standard. Compounds having IC_{50} < 5 µg/ml are considered potentially active and exposed to further in *vivo* studies.

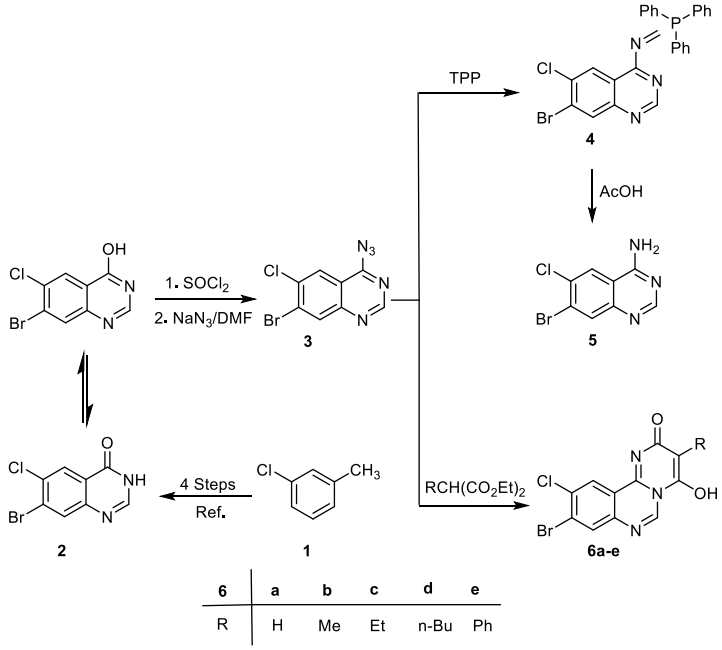
2.3. Molecular docking study.

The docking studies were created using Molecular Operating Environment (MOE-Dock) software version 2014.0901. The co-crystallized structures of EGFRWT and EGFRT790M enzymes (PDB codes: 1M17 and 3UG2) [35-37] complexed with erlotinib and gefitinib, respectively, were downloaded from the RCSB Protein Data Bank. The partial charges were automatically detected, and all minimizations for the structures were applied with MOE until an RMSD gradient of 0.05 $kcal \cdot mol^{-1}Å^{-1}$ with MMFF94x force field. Preparation of EGFRWT and EGFRT790M structures was done using Protonate 3D protocol in MOE with the default options. Validation of the docking processes was confirmed through re-docking of the original ligands, followed by docking of the targets **6b**, **6e**, **8,** and **11** into the active sites after deleting the original ligands following the reported method [38,39].

3. Results and Discussion

3.1. Chemistry.

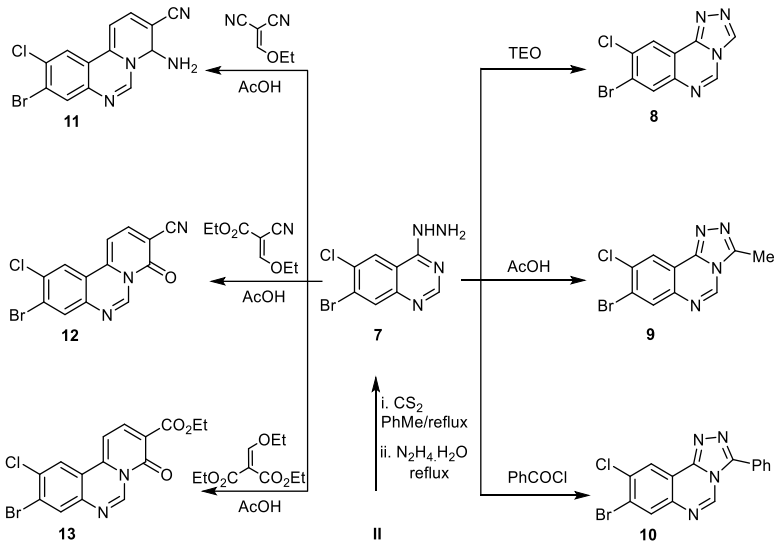
In the current investigation, the halo-substituted-4-quinazolinimine and the 2*H*-pyrimido[1,2-*c*]quinazolin-2-one compounds were synthesized starting from the key 4-azido compound. The 6,7-dihaloquinazoline-4-ol derivative **2** [40] was converted into the 4-azido-7-bromo-6-chloroquinazoline **3** in 98% yield via reaction with thionyl chloride followed by sodium azide in DMF. The ^{1}H-NMR spectrum showed three singlets at δ = 7.85, 8.30, and 8.88 ppm for Ar-H. Treating of **3** with triphenylphosphine gave **4** in 97% yield. The IR spectrum showed the characteristic band for C=N at 1609 and C=C Ar at 1568, 1426 cm^{-1}. A mixture of **4** and acetic acid was refluxed to give 7-bromo-6-chloroquinazolin-4-amine **5** in 83% yield. The IR spectrum showed the characteristic band for NH_2 at 3275, C=N at 1614, and C=C Ar at 1582, 1482 cm^{-1}. The ^{1}H-NMR spectrum showed broad singlet at δ = 7.61 for NH_2 and four singlets at δ = 7.87, 8.07, 8.46, and 7.78 for Ar-H. Treating of **3** with orthoformate derivatives afforded **6a-e** in 63-82% yields. The structures of **6a-e** were confirmed by IR, ^{1}H- and ^{13}C-NMR, mass spectroscopy as well as elemental analyses **(**Scheme 1).



Scheme 1. Synthesis of new Pyrimido[1,2-*c*]quinazolin-2-ones

The pyrido[1,2-c]quinazoline and the [1,2,4]triazolo[4,3-*c*]quinazoline systems were synthesized using the key compound 7-hydrainyl derivative **7,** which was obtained from compound **2**. Thus, treatment of the latter azide with CS_2 in toluene at reflux temperature followed by refluxing the corresponding thiol with hydrazine hydrate gave 7-bromo-6-chloro-4-hydrazinylquinazoline **7** in 85% yield, which was treated with triethylorthoformate and formic acid, acetic acid, or benzoyl chloride to give the corresponding triazoloquinazoline derivatives **8-10** in 67-73% yields, respectively.

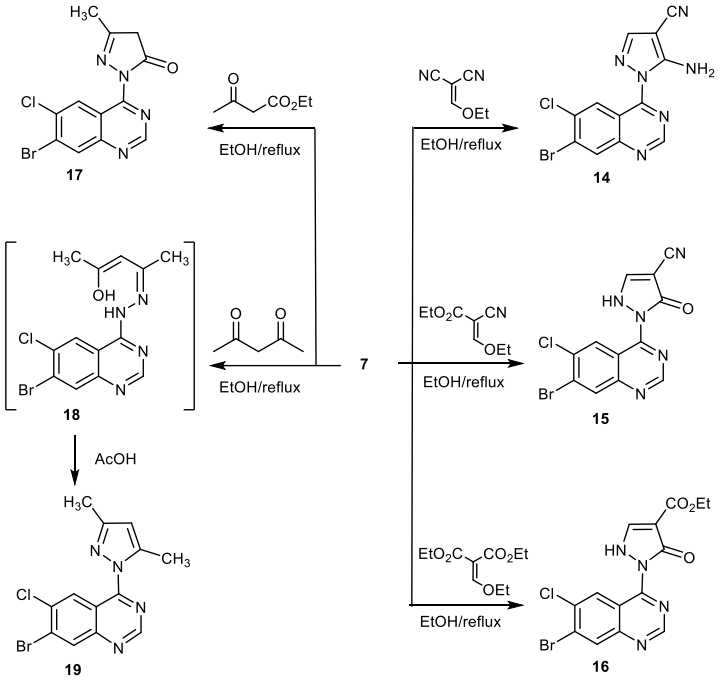
Refluxing of **7** with 2-(ethoxymethylene)malononitrile, ethyl 2-cyano-3-ethoxyacrylate, and/or diethyl 2-(ethoxymethylene)malonate in the presence of a catalytic amount from glacial acetic acid yielded **11-13** in 70-85% yields. The structures of **8-13** were confirmed by IR, ^{1}H- and ^{13}C-NMR, mass spectroscopy as well as elemental analyses (Scheme 2).



Scheme 2. Synthesis of [1,2,4]Triazolo[4,3-*c*]quinazolines and Pyrimido[1,2-*c*]quinazolines.

A mixture of **7** and ethyl 2-cyano-3-ethoxyacrylate, 2-(ethoxymethylene)malononitrile, diethyl 2-(ethoxymethylene)malonate, ethyl acetoacetate, or acetylacetone in the presence of a catalytic amount of glacial acetic acid was refluxed for 4-7 h to afford **14-19** in 66-83% yields (Scheme 3).

The ^{1}H NMR spectra of the obtained products showed the additional pyrazole protons' signals, which have also been revealed in their ^{13}C NMR spectra. In addition, their IR spectra showed the characteristic bands for the carbonitrile, the NH_2, and the carbonyl groups in their assigned structures (see Exp. part).



Scheme 3. Synthesis of new 1-(Quinazolin-4-yl)-1*H*-Pyrazoles

3.2. Cytotoxic activity.

The anticancer activity of the newly synthesized analogs was screened against human breast cancer cell line (**MCF-7)** using the assay used by Skehan and Storeng [34] to measure

the cellular membrane permeabilization (rupture) and severe irreversible cell damage. The *in-vitro* cytotoxicity evaluation was performed using doxorubicin as a reference. The obtained data were expressed as IC_{50} values that represent the compound concentrations needed to create a 50% inhibition of cell growth after 24 h of incubation, as shown in Table 1. The data showed that doxorubicin had an IC_{50} at 4–9 µM against all cells investigated with no differentiation between cancer and normal cells.

The results from Table 1 and Figure 2 explicated that some of the screened derivatives displayed an excellent to moderate inhibitory activity against the MCF-7 cancer cell line. The results showed that compounds **6b**, **6e**, **8,** and **11** revealed high anticancer activity near to that observed for doxorubicin followed by the activity of compounds **6d**, **14,** and **15**. Although being active reveals a degree of good activity, compounds **5**, **7**. **17,** and **19** showed the lowest activity results compared to the applied reference drug **(**Table 1**)**.

By correlation of the observed activity data with the characteristic structural features of the tested compounds, it has been revealed that incorporation of a condensed pyrimidine or pyridine ring, or attachment of a pyrazole ring at the quinazolyl-C^4, to the dihalo-substituted quinazoline ring system results in enhancement of the cytotoxic activity. The latter observation was achieved by the comparison of the activity of the 4-substituted quinazoline compounds (compounds **2-5** and **7**) with the prepared tricyclic derivatives. The results also showed the effect of substituents at *C*-3 (compounds **6a-e**) on the activity and revealed that the substitution of the pyrimido[1,2-*c*]quinazolines system with methyl and phenyl groups results in increased activities. Interestingly, the obtained cytotoxic activity for the prepared also indicate the effect of the nature of the substituent at the triazole-C^5 since the [1,2,4]triazolo[4,3-*c*]quinazoline, which showed that substitution with the methyl and phenyl parts lead to decreased activity compared to the structural isosteric analog (compound **8**) which is free of substitution at this position.

On the other hand, the results of the tested pyrido[1,2-c]quinazoline derivatives showed that the compound possessing the amino and the nitrile groups revealed the highest cytotoxic activity compared to those incorporating the carbonyl and ester functionalities. Furthermore, the afforded results for the quinazolin-4-yl-2,3-dihydro-1*H*-pyrazole compounds showed that the two derivatives incorporating the carbonitrile substituent at pyrazole-C^4 revealed relatively improved cytotoxic activity compared to the other derivative of the same basic ring system, which lakes for such substituent and possessing a methyl group. In addition, the lowest activity was observed by the pyrazolyl-quinazoline derivative incorporating dimethyl substituents in the attached pyrazole ring.

Table 1: The IC_{50} (µg/mL) of some of the selected new compounds against Breast cancer cell line (**MCF-7**).

Compd no.	**IC_{50} µg/ml**	**Compd no.**	**IC_{50} µg/ml**
DOX	2.97	**DOX**	2.97
3	4.50	**9**	4.00
4	4.17	**10**	3.88
5	5.00	**11**	3.45
6a	4.15	**12**	4.10
6b	3.50	**13**	4.70
6c	4.00	**14**	3.90
6d	4.90	**15**	3.90
6e	3.44	**16**	4.11
7	5.00	**17**	5.00
8	3.39	**19**	5.35

3.3. Molecular docking study.

Molecular docking is considered a vital means to predict the possible mechanisms of biologically active targets. Referring to the importance of quinazoline derivatives in the treatment of cancer through inhibition of EGFR [8-10], the promising cytotoxic derivatives **6b**, **6e**, **8,** and **11**, as representative examples of the synthesized compounds in this series, were docked into the active sites of EGFRWT and EGFRT790M enzymes.

The native ligands, erlotinib, and gefitinib were re-docked within the active sites of EGFRWT and EGFRT790M enzymes (PDB codes: 1M17 and 3UG2), respectively [36-38] to validate the docking processes and revealed energy scores -12.35 and -11.70 kcal/mol at root mean square deviation (RMDS) values of 0.91 and 1.22 Å, respectively. Regarding Figure 2, the N1 of quinazoline scaffold of erlotinib and gefitinib afforded H-bond acceptors with the backbones of **Met769** and **Met 793** within the binding sites EGFRWT and EGFRT790M enzymes (distance: 2.70 and 2.85 Å, respectively).

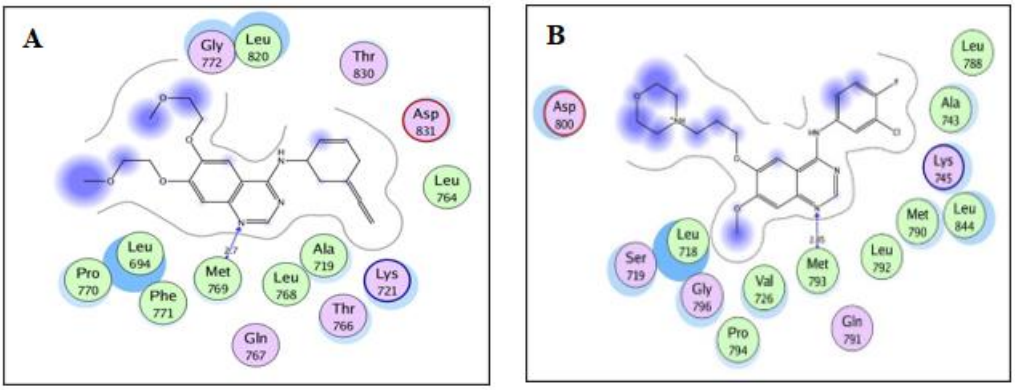
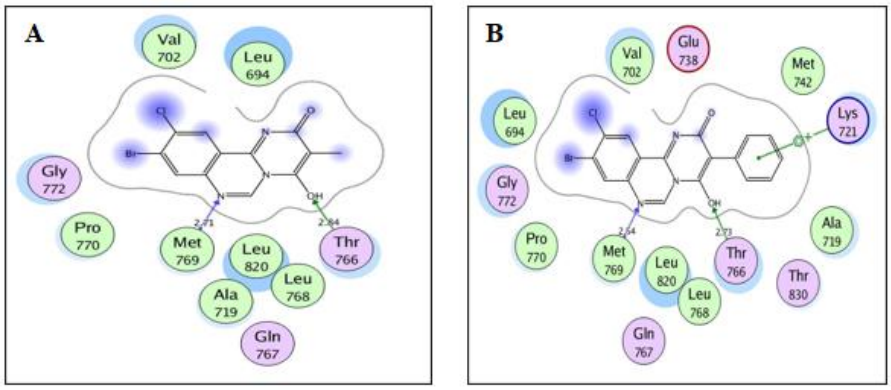


Figure 2. A & **B** views representing 2D binding modes of the native ligands erlotinib and gefitinib, into the active sites of EGFRWT and EGFRT790M enzymes (PDB codes: 1M17 and 3UG2), respectively.

As illustrated in Figure 3 and like the reference drug, erlotinib within the active site of EGFRWT, The N1 of quinazoline scaffold of the screened derivatives **6b**, **6e**, **8,** and **11** revealed H-bond acceptors with the backbone of **Met769** (distance: 2.71, 2.54, 2.69 and 2.94 Å, respectively). Moreover, the hydroxyl oxygens in compounds **6b** and **6e** gave H-bond acceptors with the sidechains of **Thr766** (distance: 2.84 and 2.73 Å, respectively). The nitrogen of the amino group in compound **11** formed H-bond acceptor with the sidechain of **Thr830** (distance: 2.96 Å). The centroid of the phenyl ring in compound **6e** exhibited arene-cation interaction with **Lys721** (Figure 3).



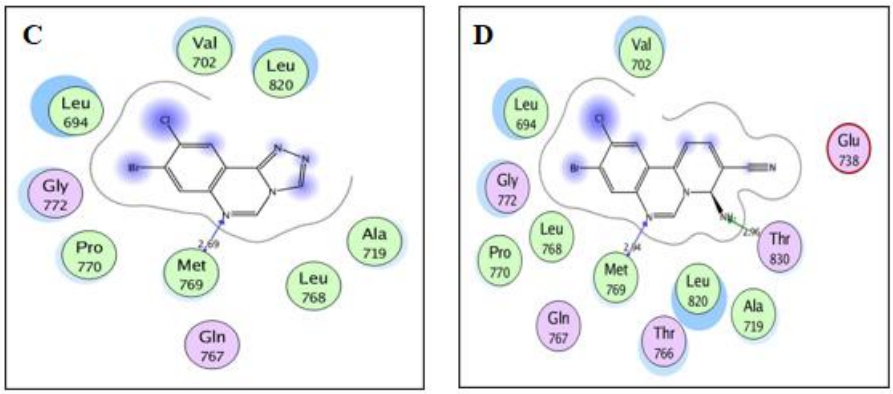


Figure 3. A-D views representing 2D binding modes of the promising targets **6b**, **6e**, **8,** and **11**, respectively, into the active site of EGFRWT enzyme (PDB codes: 1M17).

By inspection of docking within the binding site of EGFRT790M in Figure 4, the backbone of the key amino acid Met793 formed H-bonds with the N1 of quinazoline moiety in compounds 6b, 8, and 11 (distance: 3.00, 2.79, and 3.05 Å, respectively) and with the hydroxyl protons of in compounds 6b and 6e (distance: 3.15 and 2.00 Å, respectively). Furthermore, the hydroxyl proton in compound 6b afforded an H-bond donor with the backbone of Pro794 (distance: 2.09 Å).

From the previous results, it was concluded that the screened targets 6b, 6e, 8, and 11 bearing quinazoline system embedded well and nicely within the binding pockets of EGFRWT and EGFRT790M enzymes with similar interactions given by the original ligands, erlotinib and gefitinib, and that was confirmed through superimposition between them in Figure 5. Collectively, these data highlighted hybrids 6b, 6e, 8, and 11 as good leads for further optimization as promising antitumor drugs through inhibition of EGFRWT and EGFRT790M kinases.

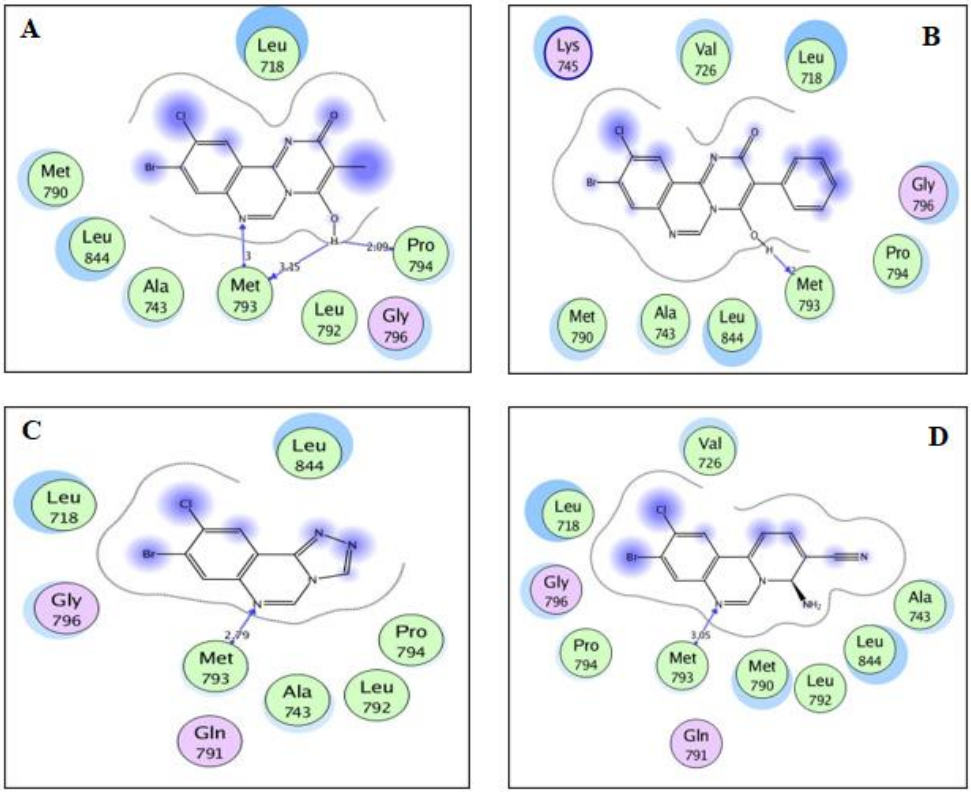


Figure 4. A-D views representing 2D binding modes of the promising targets **6b**, **6e**, **8,** and **11**, respectively, into the active site of EGFRT790M enzyme (PDB code: 3UG2).

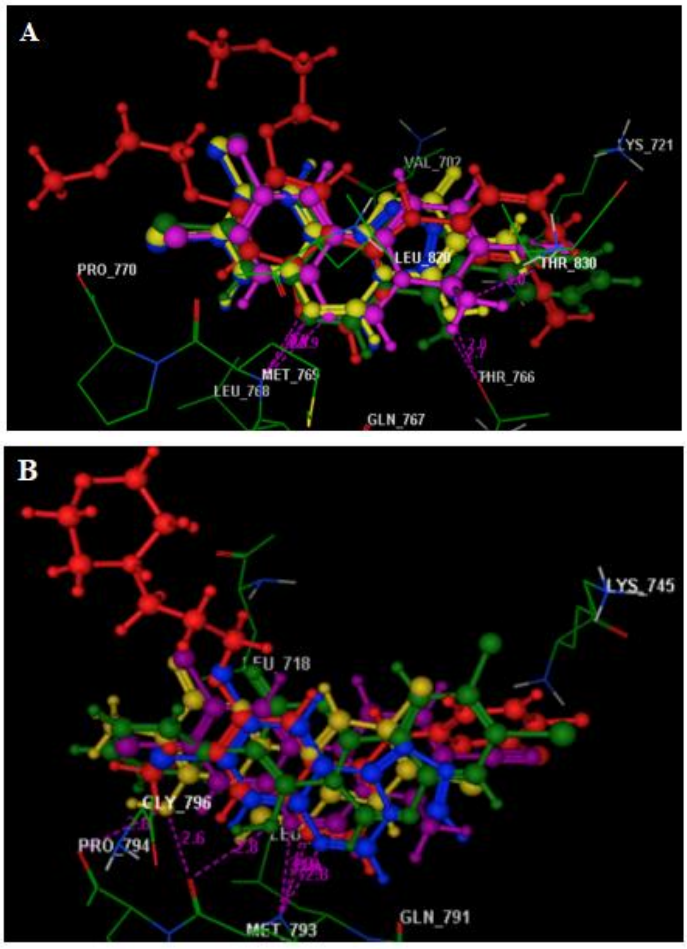


Figure 5. 3D images of the superimposition between the docked native ligands erlotinib and gefitinib (red), **6b** (yellow), **6e** (green), **8** (blue), and **11** (violet) in the active sites of EGFRWT and EGFRT790M enzymes (PDB codes: 1M17 and 3UG2), respectively.

4. Conclusions

The afforded results revealed the effect of incorporation of varied heterocyclic systems with the substituted quinazoline system and the effect of substituents in the newly synthesized 2*H*-pyrimido[1,2-*c*]quinazolin-2-one, the pyrido[1,2-c]quinazoline, the [1,2,4]triazolo[4,3-*c*]quinazoline, and the pyrazolyl-quinazoline hybrids on the anticancer activity. In addition, all the compounds incorporating the latter ring systems showed high cytotoxic effects against the human breast cancer cell line **(MCF-7),** which accounts for the importance of incorporating the quinazoline system into other heterocyclic cores such as the pyridine, pyrimidine, and pyrazole rings. The promising molecular docking findings showed that the quinazoline targets **6b**, **6e**, **8,** and **11** could be considered lead molecules to develop further novel EGFRWT and EGFRT790M inhibitors for cancer therapy.

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Conflicts of Interest

The authors declare no conflict of interest.

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